

REMARKS

Claims 1-14 are currently pending in the application. In an Office Action dated September 30, 2004 ("Office Action"), the Examiner noted that a Sequence Listing and statement under 37 CFR §1.821(f) are required, identified problems related to a previously filed Information Disclosure Statement, identified references in the Specification that need to be updated, and hyperlinks in the Specification that need to be removed, noted a potential objection to claim 9, rejected claims 1-9 under 35 U.S.C. § 112, second paragraph for being indefinite, rejected claims 13 and 14 under 35 U.S.C. § 101, rejected claims 1 and 7-9 under 35 U.S.C. § 102(b) as being anticipated by Lockhart et al., WO 97/10365, 3/20/1997 ("Lockhart"), rejected claims 1-3, 5, and 7-14 under 35 U.S.C. § 103(a) as being unpatentable over Lockhart in view of Chenchik et al., U.S. Patent No. 6,077,673 and further in view of Lewin, B., Genes IV, 1990, Oxford University Press, and rejected claims 1, 4, and 7-14 under U.S.C. § 103(a) as being unpatentable over Lockhart in view of Chenchik and further in view of Darnell et al., Molecular Cell Biology, Eds., 1990, Scientific American Books.

The Specification has been amended, and a Sequence Listing provided, to address the Examiner's requirement. Applicants' representative agrees with the Examiner's corrections to the Information Disclosure Statement, and understands that references within the current application have not been considered as prior art. The specification has been amended to remove hyperlinks, and the application referred to on page 29 is not considered by Applicants to be patentably related to the current claims. The specification has been amended to update references to applications, and to delete hyperlinks. Applicants' representative has cancelled claim 9 to address the Examiner's objection. Claim 1 has been amended to address the Examiner's indefiniteness rejection. In response to the Examiner's 35 U.S.C. § 101 rejections, claims 13 and 14 have been cancelled. Applicants' representative respectfully traverses the Examiner's 35 U.S.C. § 102(b) rejections of claims 1 and 7-9, 35 U.S.C. § 103(a) rejections of claims 1-3, 5, and 7-14, and 35 U.S.C. § 103(a) rejections of claims 1, 4, and 7-14, for reasons provided below.

Applicants' representative has carefully read the cited sections of Lockhart, but cannot find any teaching or suggestion in Lockhart for Applicants' claimed method. Please consider claim 1, provided below, with added emphasis:

1. A method for calibrating data scanned from a molecular array, the method comprising:
 - selecting a molecular array that includes a set of calibrating features containing calibrating probes *that hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*;
 - exposing the molecular array to a sample solution;
 - reading the molecular array to determine signal intensities for each feature of the molecular array;
 - calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features; and
 - calculating normalized signal intensities based on signal intensities read from features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal.

Please note the italicized language in claim 1, added to emphasize language modifying the term "calibration probe." Applicants construct a set of calibrating probes that together hybridize to a sufficient fraction of the target molecules present in sample solutions to which the array is exposed to be representative of the total concentration of target molecules in the sample solution. A basis for this claim is found in the specification beginning on line 13 of page 23:

To overcome the deficiencies of the mathematical normalization techniques and the deficiencies of common standard-feature-set techniques, embodiments of the present invention rely on determining and employing calibration feature sets containing probe molecules that reliably hybridize to large fractions of all target molecules in a wide range of sample solution types. ... An important attribute of a properly chosen calibration feature subset according to the present invention is that the average signal generated by the calibration feature subset is proportional to the total nucleic acid content of any given sample solution for which the calibrated feature subset is valid.

These passages, and the intervening textual and mathematical description of the normalization method on page 24, describe what is meant by *a sufficient fraction of target molecules in sample solutions to produce corresponding signal intensities proportional to the total concentration of target molecules in the sample solutions.*

The claimed normalization method generally does not provide accurate or useful normalization of the microarray data when the calibration probes specifically target certain gene products, or types of gene products. For example, it is common to include probes targeting a few so called "housekeeping genes" in order to measure basal transcription rates and other such parameters, but such probes do not hybridize to a large fraction of the target molecules in a sample solution. Instead they hybridize to a few, specific gene products. They are also not generally representative of the total target molecule concentration. In most cases, the majority of target gene products may experience varying degrees of differential expression, so that inferring total target concentration from the calculated concentration of a few constitutively expressed genes would be neither reasonable nor accurate. Similar considerations apply to features that contain copies of genome fragments. Such features are useful as array orientation devices, but do not necessarily hybridize to a sufficient fraction of target molecules to allow total target concentrations to be inferred. These types of features are generally used because they do usually bind some level of target molecules, and so generally produce a signal, but that is not at all the same as reliably hybridizing to a sufficient fraction of target molecules to allow total target concentration to be calculated. In general, only a very small fraction of total genome digests correspond to gene fragments, and the concentration of such gene-product-targeting probe fragments may be quite small. The currently claimed calibration probes, by contrast, reliably hybridize to a many mRNA or cRNA target molecules. As one example, the probes directed to poly(A) tails reliably hybridize to a very large fraction of human mRNA. Such probes have not been employed previously for calibration probes, because they do not distinguish individual targets or target classes, but instead hybridize relatively promiscuously to many target molecules. This was not previously considered to be an advantage.

Please note that claim 1, provided above, contains the step "calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features." The word "collective" is defined, in Webster's Third New International Dictionary, Encyclopedia Britannica, Inc., 1966 as: (1) "indicating a number of persons or things constituting one group or aggregate;" and (2) "formed by collecting : gathered into a mass, sum, or body : aggregated." The term in claim 1 is used to indicate an aggregate or combined signal intensity computed from the individual signal intensities for a set of calibrating features. One possible collective calibration intensity is the average signal intensity computed for the calibrating features, an expression for which is shown, on page 23 of the current application:

$$S_N \cong R X_N M_{NA}$$

where $N = \text{number of features in calibration subset, } \{S_{j_1}, S_{j_2}, S_{j_3} \dots S_{j_N}\}$

$$S_N = \text{average signal of subset features, } \frac{1}{N} \sum_{i=j_1}^{j_N} S_i$$

In this expression, the average signal intensity for the calibration subset is equal to the sum of the calibration-feature signal intensities $\sum_{i=j_1}^{j_N} S_i$ divided by the number of calibration features in the subset N . Other collective signal intensities are possible, including, for example, a median signal intensity, the midpoint signal intensity in an ordered list of signal intensities for the calibration features. Another possible collective signal intensity is a geometric mean, as used in the sub-expression $N \sqrt{\prod_{j=1}^N S_j}$ in the expression on page 19 of the current application. A collective signal intensity is an aggregate or combined signal intensity of the calibrating features, essentially representing, by a computed number, the aggregate signal intensity for the group of features constituting the calibration set.

Please consider the various control probes taught in Lockhart. These include normalization controls, expression-level controls, mismatch controls, and sample preparation/amplification controls. Beginning on line 26 or page 36, Lockhart describes

normalization controls as being "oligonucleotide probes that are perfectly complementary to labeled reference oligonucleotides that are added to the nucleic acid sample." In other words, the normalization controls are designed to hybridize to reference oligonucleotides with which a sample solution is spiked. Lockhart further specifies that "[t]he normalization control(s) can also be selected to reflect the (average) base composition of the other probes in the array, however in a preferred embodiment, only one or a few normalization probes are used and they are selected such that they hybridize well (i.e. no secondary structure) and do not match any target-specific probes." Clearly, by Lockhart's description, normalization probes are not intended to, and indeed do not, *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*. They do not, in fact, hybridize to target molecules of sample solutions, as clearly stated by Lockhart, but instead to reference oligonucleotides with which sample solutions are spiked. Moreover, even if normalization controls were designed to hybridize to target molecules, only a few specific normalization probes are intended to be used, as clearly stated by Lockhart, again indicating that normalization probes are not designed to hybridize to a fraction of target molecules representative of total target molecule concentration.

Lockhart also uses expression-level controls, which Lockhart describes, beginning on line 18 of page 37, as follows:

Expression level controls are probes that hybridize specifically with constitutively expressed genes in the biological sample. Expression level controls are designed to control for the overall health and metabolic activity of a cell.

Lockhart mentions a handful of housekeeping genes that provide suitable targets for expression level controls. However, Lockhart does not appear to further discuss these controls. They are certainly not intended to *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*. Instead, they are intended to provide an indication of overall cell health. Lockhart does

not suggest that a large number of expression-level controls be employed in order to obtain an indication of the total concentration of target molecules. Even were this suggestion to be made, it is unlikely that expression-level controls would serve such a purpose, since they are specifically targeted to a few, specific gene products which may not at all represent the total concentration of target molecules, in the case that many non-constitutively expressed gene products are either significantly up-regulated or down-regulated, depending on the experiment.

On page 38, Lockhart characterizes mismatch controls as follows:

Mismatch controls are oligonucleotide probes identical to their corresponding test or control probes except for the presence of one or more mismatched bases. A mismatched base is a base selected so that it is not complementary to the corresponding base in the target sequence to which the probe would otherwise specifically hybridize. One or more mismatches are selected such that under appropriate hybridization conditions (e.g. stringent conditions) the test or control probe would be expected to hybridize with its target sequence, but the mismatch probe would not hybridize (or would hybridize to a significantly lesser extent).

Moreover, Lockhart further describes mismatch controls and their use, on pages 56-57, as follows:

It is expected that after washing in stringent conditions, where a perfect match would be expected to hybridize to the probe, but not to the mismatch, the signal from the mismatch controls should only reflect non-specific binding or the presence in the sample of a nucleic acid that hybridizes with the mismatch. ... Thus, in a preferred embodiment, the signal of the mismatch probe is subtracted from the signal from its corresponding test probe to provide a measure of the signal due to specific binding of the test probe.

Clearly, mismatch controls are neither intended to, nor, in fact, *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*. Instead, they are used to detect non-specific binding, and each is used as a specific control for a specific probe.

Finally, Lockhart uses sample-preparation/amplification controls, which Lockhart describes, beginning on line 4 of page 9, as "complementary to subsequences of

control genes selected because they do not normally occur in nucleic acids of the particular biological sample being assayed."

In short, none of the control probes that Lockhart details are intended to serve, or can be considered to serve, the purpose expressly claimed for the calibrating probes in claim 1 – namely, to *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*. Lockhart's mismatch and sample-preparation/amplification controls are specifically designed not to hybridize to target molecules. Lockhart's normalization controls are designed to specifically hybridize to a few control oligonucleotides that are added to a sample solution, or, in other words, with which a sample solution is spiked. Lockhart's expression-level controls are designed to hybridize specifically to a few constitutively expressed gene products in order to represent cell health, and not as a measure of the total target-molecule concentration. Moreover, Lockhart does not teach or suggest averaging calibration probe signals and employing a normalization function. In Applicants' representative's opinion, there is nothing in Lockhart suggestive of Applicants' claimed method.

Chenchik also appears to be unrelated to the claimed method. Chenchik neither teaches nor suggests using calibrating probes that *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*. Chenchik does mention one or more additional spots of polynucleotides which are not key mouse genes in the cited passages. These also include a number of housekeeping genes to indicate basal metabolic levels, negative controls, genomic DNA, and positive controls. Chenchik teaches that these additional spots may be used to provide an indication of background or basal level of expression, orientation, and as negative controls. However, Chenchik neither teaches nor suggests the use of calibrating probes designed to *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce*

corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions. The housekeeping genes mentioned by Chenchik do not serve this purpose, because only a tiny number of housekeeping genes are mentioned, and their use is in determining basal metabolic levels and background expression levels. While these types of controls can be used to infer mRNA abundance, based on assumptions concerning the vast number of target molecules to which these types of controls do not hybridize, they do not provide a direct and/or reliable measure of mRNA abundance. Probes directed to a few housekeeping gene products do not hybridize to a sufficient fraction of the target molecules in biologically relevant sample solutions to measure total target concentration. Moreover, Chenchik does not appear to mention or suggest a normalization procedure based on calibration probes, does not mention or suggest use of average calibration probe intensities, and does not mention use of a normalization function, to all of which language in claim 1 is directed.

The Examiner's 35 U.S.C. § 102(b) rejections of claims 1 and 7-9, 35 U.S.C. § 103(a) rejections of claims 1-3, 5, and 7-14, and 35 U.S.C. § 103(a) rejections of claims 1, 4, and 7-14 all appear to primarily rely on Lockhart or Lockhart and Chenchik. But, as discussed above, neither Lockhart, alone or in combination, appear to teach, mention, or suggest Applicants' claimed invention. Applicants' representative has read both documents, and can find neither mention nor suggestion of the claimed calibration probes, or methods for employing them for normalization. In Applicants' representative's respectfully offered opinion, claim 1, and the claims that depend from claim 1, are neither anticipated nor obvious in view of Lockhart and Chenchik, and, because claim 10 includes similar language, claim 10, and claims that depend from claim 10, are also unanticipated and unobvious in view of Lockhart and Chenchik.

Lewin and Darnell appear to be cited as teaching well-known facts about poly(A) tails and Alu repeat sequences, and are otherwise unrelated to the current application. Feinberg teaches a method for radio labeling DNA fragments by solution-chemistry methods. Although the Examiner maintains, on page 16 of the Office Action, that Feinberg states "that the oligonucleotides bind to any DNA in high frequency,"

Applicants' representative can find no such statement in Feinberg. Instead, Feinberg states that "oligonucleotides can serve as primers for copying single stranded templates by a variety of DNA polymerases" (Feinberg, page 10) and that primer hexamers "have given reproducible labeling results with a wide variety of template DNA fragments" (Feinberg, page 12). Priming DNA polymerase reactions is neither related to the disclosure of the current application nor to Applicants' claimed methods. Feinberg neither teaches specifically binding of the oligomers to DNA, nor details binding constants or rate constants that would allow an inference as to whether the binding would provide stable hybridization to mRNA targets when used as probes on a microarray. They would almost certainly not exhibit the necessary binding affinities to mRNA if used as array probes.

Many of the depend claims include specific language directed to normalization details for which there is not even vaguely related disclosure in any of the cited references. For example, please consider claim 7, with emphasis added:

7. (original) The method of claim 1

wherein calculating a collective calibration signal intensity from the signal intensities read from the set of calibrating features further includes calculating a set of collective calibration signal intensities by partitioning the signal intensities generated from the set of calibrating features into sets of similar calibrating signal intensities and calculating a collective signal intensity for each set, so that the sets of similar calibrating signal intensities each covers a discrete range of signal intensities and so that the discrete ranges of signal intensities span an overall range of signal intensities generated from features of the molecular array, and

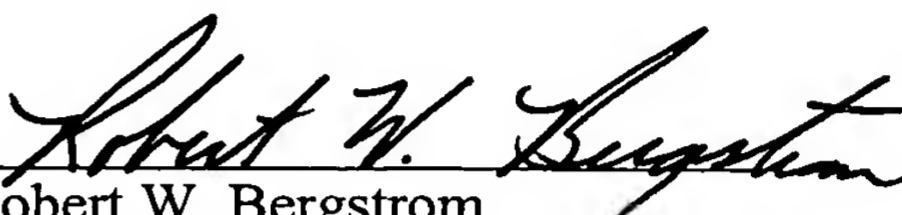
wherein calculating normalized signal intensities based on signal intensities read from features of the molecular array by applying to the signal intensities a normalization function that includes the calculated collective calibration signal further includes *applying to each signal intensity a normalization function that includes the calculated collective calibration signal calculated from the set of calibrating signal intensities within the discrete range of intensities in which the signal intensity generated from the feature of the molecular array is included.*

In none of the cited references is there any mention of a range of signal intensities, for example, or use of signal-intensity ranges in normalization. Applicants' representative respectfully observes that the broad rejection of these dependent claims is simply not supported.

Although the currently claimed methods are neither anticipated nor made obvious by Lockhart and Chenchik, Applicants' representative feels it necessary to point out that combinations of references inspired by hindsight recognition do not meet the requirements for a 35 U.S.C. § 103 obviousness-type rejection. The combination must be taught by the prior art, and not by the considered application. In the Office Action, the Examiner has attempted to combine Lockhart and Chenchik with basic facts gleaned from elementary molecular biology texts to craft a 35 U.S.C. § 103, but in Applicants' representative's respectfully submitted opinion, these combinations were inspired by hindsight recognition, and not by the cited references themselves. Just because Alu repeat sequences and poly(A) tails are well known does not allow elementary textbook descriptions of these phenomena to be combined with Lockhart and Chenchik, references that neither teach, mention, nor suggest the currently claimed methods, to suggest that calibration probes specifically based on the wide occurrence of poly(A)-tails and Alu-repeat-sequences in human mRNA are obvious. These specific calibration probes were chosen because Applicants' method needs calibration probes that *hybridize to a sufficient fraction of target molecules in sample solutions to which the molecular array is intended to be exposed to produce corresponding signal intensities upon reading of the calibrating probes proportional to the total concentration of target molecules in the sample solutions*. The cited references neither teach nor suggest this class of calibration probes. Even had the references taught or suggested instances of this class of calibration probes, which they do not, a new instance of the class would be patentable if the that instance had not yet been employed or suggested as a calibration probe.

All of the claims remaining in the application are now clearly allowable.
Favorable consideration and a Notice of Allowance are earnestly solicited.

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Enclosures:

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